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CHARACTERISTICS OF AN AMINOHYDROLASE DISTINCT FROM ADENOSINE DEAMINASE IN CULTURED HUMAN LYMPHOBLASTS

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Summary

An inherited deficiency of adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) is associated with an autosomal recessive form of severe combined immunodeficiency disease. Affected patients exhibit markedly reduced or absent adenosine deaminating activity in various tissues. In this study we have demonstrated the presence of a low level aminohydrolase activity in 11 different normal and adenosine deaminase-deficient lymphoblast cell lines which is apparently distinct from normal adenosine deaminase. Based on enzymatic, physical and immunoreactive properties, this lymphoblast aminohydrolase does not appear to be related to adenosine deaminase and is most likely coded for by a different gene locus. In future investigations designed to characterize mutant forms of adenosine deaminase, it will be important to distinguish this lymphoblast aminohydrolase activity from putative products of the adenosine deaminase gene locus.

Introduction

Adenosine deaminase (adenosine aminohydrolase, EC 3.5.4.4) catalyzes the conversion of either adenosine or deoxyadenosine to produce inosine and deoxyinosine, respectively, with the liberation of ammonia. The discovery of a deficiency of adenosine deaminase in some patients with an autosomal recessive form of severe combined immunodeficiency disease has provided an important clue to the pathogenesis of immune dysfunction at the molecular level [1—6].

Abbreviations EHNA, *erythro*-9-(2-hydroxy-3-nonyl)adenine

A characterization of the residual adenosine deaminase activity in patients with adenosine deaminase deficiency and severe combined immunodeficiency disease has been difficult due to the extremely limited amount of enzyme activity expressed in various tissues of these patients. Further, any interpretation of the nature of the genetic heterogeneity of adenosine deaminase in these patients could be complicated without a complete understanding of the molecular and electrophoretic heterogeneity of the enzyme expressed in normal human tissues [7—10]. Recent studies have shown that human adenosine deaminase exists either as a 'particulate' species or as one of two soluble molecular forms, designated small form (M_r 38 000) and large form (M_r 298 000), which are interconvertible [10]. The small form of the enzyme from human erythrocytes is a single polypeptide exhibiting multiple electrophoretic forms arising from post-translational modification of a single gene product. In part, this heterogeneity may be due to sulfhydryl group modification on the enzyme [11—15]. The large molecular form of adenosine deaminase (M_r 298 000) is composed of two molecules of small-form adenosine deaminase (M_r 38 000) and one molecule of a specific adenosine deaminase-binding protein (M_r 213 000) (also termed conversion factor or complexing protein) [16—18]. The large form of the enzyme also exists as 'tissue specific' electrophoretic variants. These variants appear to be generated by heterogeneity in the carbohydrate portion of the binding protein and are not due to additional variation in the small-form deaminase present in the complex [19].

Early studies of adenosine deaminase molecular heterogeneity indicated the presence of a low level of an 'intermediate' form of the enzyme (M_r 110 000) in a variety of human tissues [10]. In addition, analysis of splenic tissue from a patient with adenosine deaminase deficiency and severe combined immunodeficiency disease revealed that the molecular form of adenosine deaminase was exclusively the 'intermediate' species [20]. Subsequently, Schrader et al. [21] demonstrated that an 'aminohydrolase' intermediate in molecular weight between large- and small-form adenosine deaminase existed in normal spleen as well as in splenic tissue of another patient with adenosine deaminase deficiency and immune dysfunction. This splenic 'aminohydrolase' appeared to be different from normal adenosine deaminase based on K_m value, immunoreactivity, pH optimum and insensitivity to the potent adenosine deaminase inhibitor, EHNA. It has not been established, however, whether this low level of 'aminohydrolase' activity studied in these two subjects (a) arose from adenosine deaminase degradation during post-mortem tissue autolysis, (b) represented a modified product of the adenosine deaminase gene locus, or (c) was a universal finding in viable human cells.

In this study, we have demonstrated the presence of an aminohydrolase in 11 different normal and adenosine deaminase-deficient B-lymphoblast cell lines which appears similar to the splenic aminohydrolase. An analysis of the enzymatic, physical and immunoreactive properties of the lymphoblast aminohydrolase suggests that this enzyme is distinct and not related to adenosine deaminase

Materials and Methods

[8-^{14}C]Adenosine (59 mCi/mmol) and [U-^{14}C]deoxyadenosine (500 mCi/mmol) were obtained from Amersham/Searle *erythro*-9-(2-Hydroxy-3-nonyl) adenine (EHNA) was a generous gift from Burroughs Wellcome, while 2'-deoxycoformycin was generously provided by Parke Davis, A Division of Warner/Lambert. The adenosine deaminase-binding protein was purified to homogeneity from human kidney as previously described [17]. All other chemicals and reagents used were of the highest quality commercially available. Normal B-lymphoblast cell lines (GM 130, GM 131, GM 333, GM 621, GM 1078 and MGL-8) and adenosine deaminase-deficient B-lymphoblast cell lines (GM 2471, GM 2606, GM 2294, GM 2445 and GM 2756 transformed from lymphocytes of patients with adenosine deaminase deficiency) were obtained from the Human Genetic Mutant Cell Repository, Camden, NJ.

All lymphoblast cell lines were cultured in RPMI medium supplemented with 10% adenosine deaminase-deficient fetal calf serum. Adenosine deaminase-deficient fetal calf serum was prepared by passing unaltered fetal calf serum (Grand Island Biological Co.) through an anti-adenosine deaminase-IgG affinity column, prepared as previously described [22]. Fetal calf serum recovered as the column filtrate had less than 1% of the original adenosine deaminase present in untreated fetal calf serum and had no detectable 'aminohydrolase' activity. Cultured lymphoblasts were harvested in log phase of growth, washed twice with phosphate-buffered saline (50 mM $NaHPO_4$, pH 7.4/154 mM NaCl), and lyzed by repeated freeze-thawing. The crude cell extracts were dialyzed 1—1000 (v/v) in 10 mM Tris-HCl, pH 7.4/154 mM NaCl (buffer A) Membrane-free cell extracts were prepared by centrifugation of crude cell lysates for 20 min at 100 000 × *g* in a Beckman airfuge.

Adenosine deaminating activity in lymphoblast extracts was assayed by a radiochemical technique previously described [20]. Assay conditions utilized [8-^{14}C]adenosine (2 mCi/mmol) at a final adenosine concentration of 4 mM (high adenosine reaction mixture). A similar reaction mixture was also prepared using [U-^{14}C]deoxyadenosine as substrate.

The molecular forms of adenosine deaminating activity in lymphoblast cell extracts were analyzed and isolated by sucrose gradient ultracentrifugation, using a previously described technique [10]. Cell extracts were applied to isokinetic sucrose gradients (10—28.2%) and centrifuged for 40h at 34 000 rev./min using a Spinco SW41 rotor in a Beckman Model L5-50 ultracentrifuge. Sucrose gradients were fractionated and assayed for adenosine deaminating activity using the high adenosine concentration reaction mixture. Fractions containing adenosine deaminating activity were pooled, dialyzed in buffer A (1—1000, v/v) and concentrated on a Diaflo ultrafiltration system with a PM-10 membrane (Amicon Corp., Lexington, MA).

The procedures for the determination of apparent substrate specificity, pH optimum, K_m value and heat stability of adenosine deaminating activity have been previously reported [10,15]. Molecular weights were determined by sucrose gradient ultracentrifugation using the method of McCarty et al. [23].

The concentration and relative affinity of immunoreactive adenosine deaminase protein and the adenosine deaminase-binding protein in lymphoblast

cell extracts was quantified using previously described radioimmunoassay techniques [22,24]. Protein concentration was determined by the method of Lowry et al. [25] using bovine serum albumin as standard.

Results

Extracts from a normal B-lymphoblast cell line (MGL-8) and an adenosine deaminase-deficient B-lymphoblast cell line (GM 2445) were analyzed for adenosine deaminating activity after sucrose gradient ultracentrifugation. As shown in Fig. 1A, when sucrose gradient fractions were assayed with a high concentration of adenosine in the reaction mixture, as described in Materials and Methods, a predominant peak of adenosine deaminating activity was observed at $s_{20,w}$ 3.8 S (fraction 28) similar to normal small-form adenosine deaminase (M_r 38 000) [10,15]. In addition, a minor peak of adenosine deaminating activity was also present at an $s_{20,w}$ of 7.4 S (fraction 15). When the sucrose gradient fractions were assayed for adenosine deaminating activity in the presence of 200 μM EHNA, enzyme activity in the predominant peak ($s_{20,w}$ 3.8 S) was completely inhibited, while greater than 90% of the activity in the smaller peak ($s_{20,w}$ 7.4 S) was retained (Fig, 1A). A similar analysis of the adenosine deaminase-deficient B-lymphoblast cell line, GM 2445, (Fig. 1B) revealed that virtually all of the adenosine deaminating activity present in this cell line exhibited an $s_{20,w}$ of 7.4 S and was insensitive to inhibition with EHNA. When the activity with an $s_{20,w}$ of 7.4 S was isolated from either normal or adenosine deaminase-deficient lymphoblast cell extracts by sucrose gradient

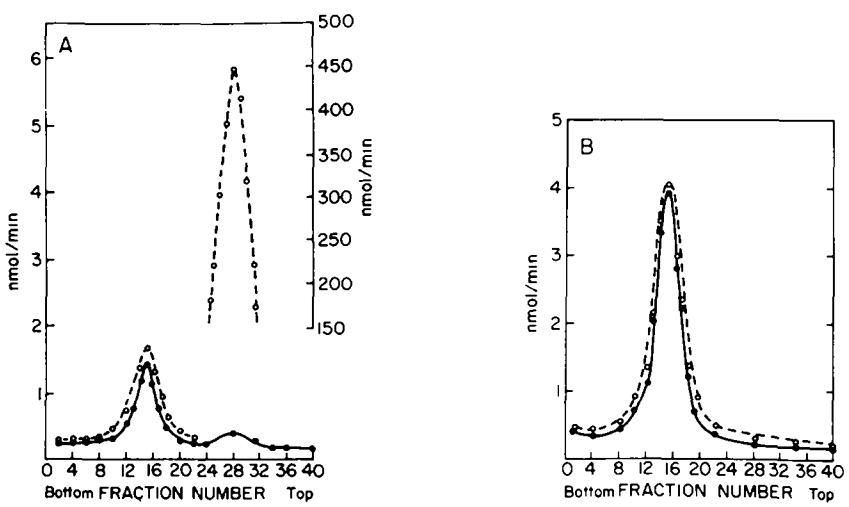

Fig 1 Sucrose density ultracentrifugation of adenosine deaminating activity in human B-lymphoblast cell extracts Cell extracts were prepared by freeze-thawing cells five times followed by centrifugation at 100 000 × *g* The soluble preparation was then applied to an isokinetic sucrose gradient (10—28,2%) and centrifuged at 4°C for 40 h at 34 000 rev /min with a Spinco SW41 rotor in a Beckman model L5-50 ultracentrifuge Fractions were collected and assayed for adenosine deaminating activity using [^{14}C]-adenosine (2 mCi/mmol, 4 mM) reaction mixture (○———○) and reaction mixture containing 200 μM EHNA (●———●) A Normal B-lymphoblast cell extract, MGL-8 B Adenosine deaminase-deficient B-lymphoblast cell extract, GM 2445

TABLE I

ADENOSINE DEAMINATING ACTIVITY IN NORMAL AND ADENOSINE DEAMINASE-DEFICIENT B-LYMPHOBLAST CELL LINES

Number of control cell lines indicated in parenthesis Mean ± 1 S D based on at least three determinations of each cell line Total adenosine deaminating activity (ADA) in lymphoblast cell extracts was assayed using [^{14}C]adenosine (2 mCi/mmol) at a final concentration of 4 mM EHNA-insensitve adenosine deaminating activity was determined using 4 mM [^{14}C]adenosine (2 mCi/mmol) with 200 μM EHNA Adenosine deaminase activity was considered to represent the difference of the total activity and the EHNA-insensitive aminohydrolase activity

Lymphoblast cell lines	Adenosine deaminating activity (nmol/min per mg)		
	Total	EHNA-insensitive aminohydrolase	Adenosine deaminase (Total minus EHNA-insensitive activity)
Normal (6)	72 69 ± 15 48	0 26 ± 0 17	72 43
ADA-deficient			
GM 2294	25 30 ± 1 14	0 58 ± 0 02	24 72
GM 2756	0 53 ± 0 02	0 49 ± 0 02	0 04
GM 2471	0 40 ± 0 01	0 29 ± 0 01	0 11
GM 2606	0 33 ± 0 02	0 32 ± 0 01	0 01
GM 2445	2 00 ± 0 10	1 93 ± 0 05	0 07

ultracentrifugation, dialyzed and reapplied to a further sucrose gradient, the enzyme activity was invariably present as a single peak with an $s_{20,w}$ of 7.4 S. This EHNA-insensitive aminohydrolase activity showed no evidence of dissociation to a species of $s_{20,w}$ 3.8 S.

Additional cultured B-lymphoblast cell lines from normal and adenosine deaminase-deficient subjects were analyzed for adenosine deaminating activity. As shown in Table I, the level of total adenosine deaminating activity in adenosine deaminase-deficient B-lymphoblast cell lines varied from 0 5 to 35% of normal. In four of the five mutant cell lines studied, the vast majority of the adenosine deaminating activity present was the EHNA-insensitive aminohydrolase. In addition, the level of EHNA-insensitive aminohydrolase activity in four of the five mutant cell lines overlapped the range noted in six cell lines derived from adenosine deaminase positive individuals.

Normal lymphoblast adenosine deaminase (small-form, $s_{20,w}$ 3 8 S) isolated from sucrose gradients was incubated with a 100-fold molar excess of adenosine deaminase-binding protein, and subjected to sucrose gradient ultracentrifugation. The resulting enzyme activity profile revealed complete conversion of the small-form adenosine deaminase to the large molecular form of the enzyme ($s_{20,w}$ 10.0 S) (Fig. 2A). Under the conditions of this experiment, no detectable EHNA-insensitive aminohydrolase activity was observed. Using the same experimental conditions, lymphoblast EHNA-insensitive aminohydrolase ($s_{20,w}$ 7 4 S) isolated from sucrose gradients was also incubated with a 100-fold excess of adenosine deaminse-binding protein. The EHNA-insensitive aminohydrolase isolated from either normal or adenosine deaminase-deficient lymphoblasts could not be converted to a species of higher $s_{20,w}$ as observed with normal small-form adenosine deaminase (Fig. 2B).

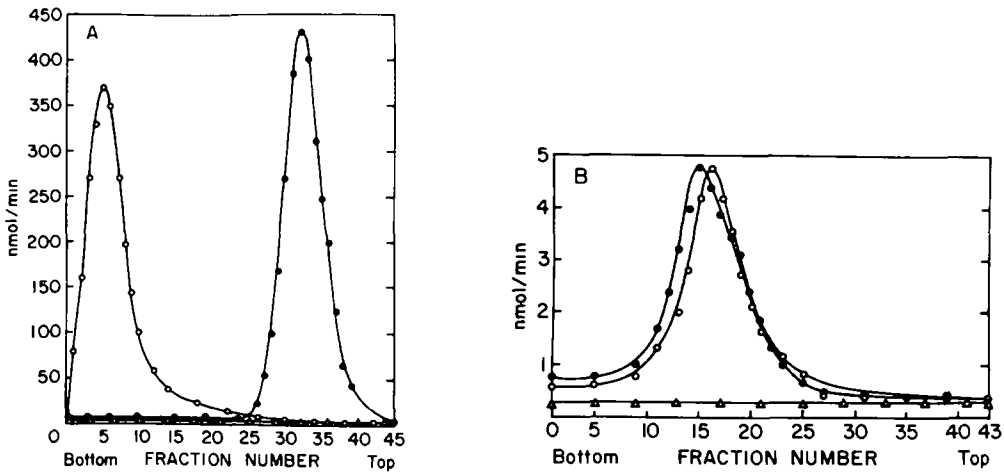

Fig 2 Interaction of adenosine deaminase and the EHNA-insensitive aminohydrolase with adenosine deaminase-binding protein A Lymphoblast adenosine deaminase isolated from sucrose gradients (3200 nmol/min) was incubated for 20 min at 37°C with 0 5 mg bovine serum albumin (●———●) or with 0 5 mg purified adenosine deaminase-binding protein prepared as described in Methods (○———○) Purified adenosine deaminase-binding protein was incubated alone as above (△———△) B Lymphoblast EHNA-insensitive aminohydrolase isolated from sucrose gradients (35 nmol/min) was incubated as above with 0 5 mg bovine serum albumin (●———●) or with 0 5 mg adenosine deaminase-binding protein (○———○) Purified adenosine deaminase-binding protein was incubated alone (△———△) All incubation mixtures were centrifuged into separate sucrose gradients

The physical and kinetic properties of the EHNA-insensitive aminohydrolase isolated from normal and adenosine deaminase-deficient lymphoblast cell lines and normal small-form lymphoblast adenosine deaminase are summarized in Table II. The EHNA-insensitive aminohydrolase has an $s_{20,w}$ of 7.46 ± 0.18 S (mean ±S.D., 11 determinations) with a calculated mean molecular weight of 110 000, while small-form adenosine deaminase has an $s_{20,w}$ of 3.8 ± 0.02 S (mean ±S.D., five determinations) with a calculated mean molecular weight of 38 000. The EHNA-insensitive aminohydrolase has; (a) a sharp pH optimum of 5.5—6.0 as compared to the higher broad pH optimum of adenosine deaminase,

TABLE II

CHARACTERISTICS OF THE MOLECULAR FORMS OF ADENOSINE DEAMINATING ACTIVITY IN LYMPHOBLASTS

Extracts from normal lymphoblasts and adenosine deaminase-deficient lymphoblasts were subjected to sucrose gradient ultracentrifugation Peak fractions corresponding to the EHNA-insensitive aminohydrolase were isolated as described in Methods Adenosine deaminase was isolated from normal lymphoblasts in a similar manner Numbers in parenthesis indicate the number of determinations All other values reported represent the average of two determinations

Property	EHNA-insensitive aminohydrolase	Adenosine deaminase
Sedimentation coefficient (1 10^{-13} S)	7 46 ± 0 18 (11)	3 8 ± 0 02 (5)
Estimated molecular weight (from $S_{20,w}$)	110 000	38 000
pH Optimum	5 5—6 0	6—8
K_m value		
adenosine	3 06 mM	0 065 mM
deoxyadenosine	0 72 mM	0 075 mM
Relative substrate specificity deoxyadenosine/adenosine	0 1	1 0

(b) a markedly elevated K_m value for adenosine (3.06 mM) and deoxyadenosine (0.72 mM) as compared to the K_m value of adenosine deaminase with these two substrates (0.065 and 0.075 mM, respectively), and (c) a lower rate of catalytic activity with the substrate deoxyadenosine, as compared to that with adenosine, than noted with adenosine deaminase.

The heat stability of adenosine deaminating activity is shown in Fig. 3. Under the conditions of our study, adenosine deaminase exhibited a logarithmic decay of enzyme activity at 68°C with a $t_{1/2}$ of approx. 30 min, while the EHNA-insensitive aminohydrolase activity was reduced by less than 5% over the 90 min incubation period. During the time course of adenosine deaminase heat inactivation, no EHNA-insensitive aminohydrolase activity was generated.

The effect of divalent cations on adenosine deaminating catalytic activity is shown in Table III. Of the divalent cations tested, only iron and nickel selectively inhibited normal adenosine deaminase.

The relative substrate specificity of the EHNA-insensitive aminohydrolase was compared to adenosine deaminase as shown in Table IV. The potential substrates, AMP, dAMP, ADP, dADP, ATP, dATP, cytidine, cytosine, deoxycytidine, CMP, dCMP, CDP, dCDP, CTP, dCTP, guanine, guanosine, deoxyguanosine, uridine and deoxyuridine, did not compete with the conversion of adenosine to inosine whether catalyzed by adenosine deaminase or the EHNA-insensitive aminohydrolase under the conditions of our study. However as shown in Table IV, adenine, 6-chloropurine and 2,6-diaminopurine reduced the deamination of adenosine to inosine when catalyzed by the EHNA-insensitive aminohydrolase while *N*-carbamyl-β-alanine and 6-methylaminopurine riboside reduced the deamination of adenosine when catalyzed by adenosine deaminase. A further differential effect was observed with the competitive adenosine deaminase inhibitors. While the EHNA-insensitive aminohydrolase was relatively insensitive to the adenosine deaminase inhibitor, EHNA, it was completely inhibited by 2′-deoxycoformycin similar to adenosine deaminase. Using

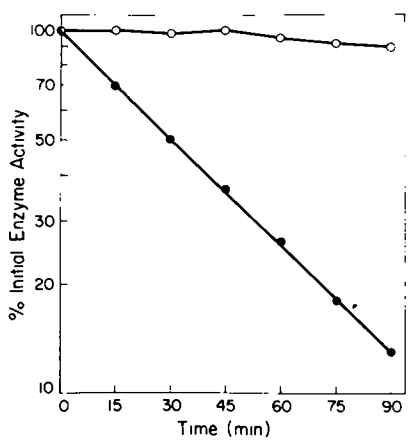

Fig 3 Heat stability of adenosine deaminating activity Lymphoblast cell lysates (protein concentration, 2 mg/ml) were incubated at 68°C in buffer A for various times Extract of MgL-8 containing normal adenosine deaminase (●———●) Extract of GM 2445 containing exclusively EHNA-insensitive aminohydrolase activity (○———○) (see Fig 1B)

TABLE III

SENSITIVITY OF LYMPHOBLAST ADENOSINE DEAMINATING ACTIVITY TO INHIBITION BY DIVALENT CATIONS

All divalent cations were used as their chloride salts at a final concentration of 2 5 mM The EHNA-insensitive aminohydrolase and adenosine deaminase were isolated from sucrose gradients and were assayed using K_m concentrations of adenosine in the presence of divalent cations The EHNA-insensitive aminohydrolase and adenosine deaminase were assayed using [8-^{14}C]adenosine (2 μCi/μmol) at final adenosine concentrations of 4 mM and 0 1 mM, respectively Results are expressed as a percentage of inhibition compared to the control with only buffer added

Cation	Percent inhibition of adenosine deaminating activity	
	EHNA-insensitive aminohydrolase	Adenosine deaminase
Iron	0	71
Nickel	4	76
Magnesium	16	10
Manganese	23	48
Calcium	25	18
Mercury	94	97
Cadmium	76	96
Cobalt	30	56
Copper	96	98
Barium	14	5
Zinc	93	98

TABLE IV

APPARENT SUBSTRATE SPECIFICITY OF LYMPHOBLAST ADENOSINE DEAMINATING ACTIVITY

The final concentration of each substrate tested was 2 5 mM with the exception of EHNA and 2′-deoxycoformycin which were used at final concentrations of 200 μM and 20 μM, respectively All solutions were prepared in 50 mM Tris HCl, pH 7 4, on the day of the study The EHNA-insensitive aminohydrolase and adenosine deaminase were isolated from sucrose gradients and were assayed using K_m concentrations of adenosine in the presence of various compounds The EHNA-insensitive aminohydrolase and adenosine deaminase were assayed using [8-^{14}C]adenosine (2 μCi/μmol) at final adenosine concentrations of 4 mM and 0 1 mM, respectively Results are expressed as a percentage of inhibition compared to the control with only buffer added

Compound	Percent inhibition of deaminating activity	
	EHNA-insensitive aminohydrolase	Adenosine deaminase
Adenine	75	51
N-Carbamyl-β-alanine	0	78
6-Methylaminopurine riboside	42	88
4-Amino-5-imidazole carboxamide-HCl	47	42
6-Chloropurine riboside	18	24
6-Chloropyrine	70	15
6-Methyl mercaptopurine riboside	25	55
Cordycepin (3′-deoxyadenosine)	51	90
2,6-Diaminopurine	99	8
5-Adenosyl homocysteine	12	8
Homocysteine thiolactone	9	7
EHNA	8	98
2′-Deoxycoformycin	99	99

specific radioactive-labeled substrates and established radiochemical assay procedures, neither the EHNA-insensitive aminohydrolase nor adenosine deaminase were able to catalyze the deamination of guanine, adenine, AMP or cytidine.

The possibility that this EHNA-insensitive aminohydrolase activity reflected an aggregated or denatured form of adenosine deaminase was examined further by a radioimmunochemical assay. Equivalent activities of lymphoblast adenosine deaminase and EHNA-insensitive aminohydrolase, 10, 5.0 and 2.5 nmol/min per ml of each respectively, were assayed with a previously described competitive adenosine deaminase radioimmunoassay [24]. Under the conditions of this assay, the least detectable quantity of adenosine deaminase was equivalent to 0.17 nmol/min per ml or 0.3 ng/ml. Using the radioimmunoassay, the dilutions of lymphoblast adenosine deaminase showed a normal competition for antibody binding similar to the adenosine deaminase standard used in the assay (slope −1.0) and were found to be completely cross-reactive (greater than 99%). When the same dilutions of EHNA-insensitive aminohydrolase activity were assayed, virtually undetectable levels of adenosine deaminase protein (i.e., less than 1% immunoreactive material relative to normal adenosine deaminase) were observed. In a similar manner both lymphoblast adenosine deaminase and the EHNA-insensitive aminohydrolase were assayed for the presence of the adenosine deaminase-binding protein using a previously described radioimmunoassay technique [22]. In both cases the level of adenosine deaminase-binding protein was virtually undetectable (less than 0.5 ng/ml). These data suggested that the EHNA-insensitive aminohydrolase did not share any antigenic determinants in common with normal adenosine deaminase and further was not associated or complexed with the adenosine deaminase-binding protein in lymphoblast cell lines.

Discussion

In this study we have demonstrated the presence of an EHNA-insensitive aminohydrolase in both normal and adenosine deaminase-deficient B-lymphoblast cell lines which is similar if not identical with a previously reported splenic 'aminohydrolase' [21]. Further characterization of the enzymatic, physical, and immunoreactive properties of this aminohydrolase suggests that this enzyme is not related to adenosine deaminase and is most probably coded for by a different gene locus than adenosine deaminase.

Analysis of adenosine deaminating activity in both normal and adenosine deaminase-deficient lymphoblast cell lines revealed the presence of low but similar levels of EHNA-insensitive aminohydrolase activity. With the exception of cell line GM 2294, the adenosine deaminating activity in all adenosine deaminase-deficient cells was almost completely EHNA-insensitive indicating the presence of an EHNA-insensitive aminohydrolase similar to that reported by Schrader et al. [21] in spleen. Cell line GM 2294, with 35% of normal adenosine deaminating activity, was obtained from an immunologically normal, healthy subject with severe erythrocyte adenosine deaminase deficiency [26]. However, even in this patient's lymphoblasts, the level of the EHNA-insensitive aminohydrolase was still comparable to that in all other adenosine deaminase

deficient as well as the normal cell lines analyzed. These data suggest that the level of adenosine deaminase activity does not apparently regulate the level of the EHNA-insensitive aminohydrolase activity in lymphoblasts.

The EHNA-insensitive aminohydrolase present in both normal and adenosine deaminase-deficient B-lymphoblast cell lines appears to be similar if not identical to the 'aminohydrolase' activity described in spleen. Schrader et al. [21], reported that the splenic 'aminohydrolase' had an estimated molecular weight of 92 000 and did not appear to cross-react with anti-adenosine deaminase serum (immobilized to Sepharose). In our studies we have demonstrated that the lymphoblast EHNA-insensitive aminohydrolase has a molecular weight of 110 000 and showed less than 1% cross-immunoreactivity relative to normal adenosine deaminase. Further, it did not contain an adenosine deaminase-binding protein component (less than 1%) using sensitive and specific radioimmunoassays. The pH optimum (6.5), K_m values for adenosine (2 mM), insensitivity to EHNA, and inhibition by adenine reported for the splenic 'aminohydrolase' compare well to our findings for the lymphoblast EHNA-insensitive aminohydrolase. In addition, we have examined other properties of this lymphoblast aminohydrolase which would serve to distinguish it from adenosine deaminase such as, its K_m value for deoxyadenosine, sensitivity to heavy metals, heat stability, relative substrate specificity, interaction with the adenosine deaminase-binding protein, and sensitivity to another potent competitive adenosine deaminase inhibitor, deoxycoformycin.

Relative to adenosine deaminase, the EHNA-insensitive aminohydrolase activity in all lymphoblast cell lines had a high K_m value for the substrates adenosine and deoxyadenosine suggesting that this enzyme might be more active with other more specific substrates. However, in our study of apparent substrate specificity only selected purine bases were found to markedly compete with the substrate adenosine and of those compounds tested, none appeared to be deaminated. With respect to adenosine and purine metabolism in general, we found that the EHNA-insensitive aminohydrolase isolated from sucrose density gradients did not deaminate guanine, cytidine, AMP or adenine nor did it have enzymatic activity of S-adenosyl homocysteine hydrolase. The functional significance of this aminohydrolase thus still remains to be established.

We have considered the possibility that this low level of EHNA-insensitive aminohydrolase activity in lymphoblasts may be related to adenosine deaminase and may possibly represent denaturated or aggregated product(s) of normal adenosine deaminase protein degradation. However, the level of the EHNA-insensitive aminohydrolase activity in 11 different, actively growing lymphoblast cell lines was apparently independent of the level of adenosine deaminase (see Table I). In addition heat denaturation of normal adenosine deaminase failed to produce EHNA-insensitive aminohydrolase type activity. Further, the EHNA-insensitive aminohydrolase showed less than 1% cross-immunoreactivity relative to normal adenosine deaminase and failed to show any interaction with the adenosine deaminase-binding protein. These data taken together strongly suggest that the lymphoblast aminohydrolase is not related to adenosine deaminase and is coded for by a different gene locus than that coding for adenosine deaminase.

The presence of this low level of EHNA-insensitive aminohydrolase activity described in lymphoblasts may be a universal finding in a variety of normal human tissues. Therefore, in future investigations designed to characterize the low level of residual adenosine deaminase in patients with adenosine deficiency and severe combined immunodeficiency disease, it will be important to either exclude the presence of this EHNA-insensitive aminohydrolase activity or be able to distinguish it from a putative altered product of the adenosine deaminase gene locus. In our study we have presented several distinguishing characteristics of the EHNA-insensitive aminohydrolase and adenosine deaminase for this purpose.

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